

Yields in biomass and chemical constituents of four commercially important marine microalgae with different culture media

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Abstract

The use of the commercial nutrient Algal-1 was studied in mass cultures of the marine microalgae *Tetraselmis suecica*, *Dunaliella tertiolecta*, *Isochrysis galbana* and *Phaeodactylum tricornutum*, in comparison with the use of media such as ES-Provasoli, and Walne medium.

Maximum cell concentrations were always found in Algal-1 cultures and these values were always significantly higher than those obtained in the remaining media. The cellular density obtained in Algal-1 cultures was between 59% and 79% higher than that obtained in the traditional culture media used for *T. suecica*, between 70% and

109% for *D. tertiolecta*, between 33% and 60% for *I. galbana* and between 63% and 105% for *P. tricornutum*.

The culture medium used affected the chemical composition of microalgal cells. Protein is the major cellular component for all the species assayed and with all the media proved. Maximum values of protein/cell were obtained in Algal-1 cultures, except for *P. tricornutum*, whereas maximum values of reserve products (carbohydrates and lipids) never occurred in Algal-1 cultures, except for *T. suecica*. Differences in the protein content were higher than those found in the other cellular constituents.

Maximum values of protein, lipids and carbohydrates per liter of culture always occurred in Algal-1 cultures, with significant differences in the values obtained in the remaining cultures. Maximum protein concentration (mg/liter) obtained in Algal-1 cultures was between 155% and 334% higher than that obtained in the remaining media for *T. suecica*, between 75% and 194% for *D. tertiolecta*, between 75% and 195% for *I. galbana* and between 93% and 225% for *P. tricornutum*. Daily protein yields (mg liter/day) also occurred in Algal-1 cultures.

Ratios between protein values per liter of culture reached in each traditional medium and those reached in Algal-1 show values between 0.2 and 0.6 for protein content; therefore, Algal-1 cultures produced between 2 and 5 times more protein than the remaining cultures. Ratios for carbohydrates and lipids were between 0.4 and 1.0, and differences between traditional culture media and Algal-1 were generally lower than those obtained for proteins.

Results showed better yields in Algal-1 cultures than those obtained with these traditional culture media.

Indexed keywords

Engineering controlled terms: Algae - Agricultural Applications; Biomass - Production; Carbohydrates - Biosynthesis; Lipids - Biosynthesis; Proteins - Biosynthesis

Engineering uncontrolled terms: Algae Culture; Marine Microalgae

Engineering main heading: Aquaculture

Species Index: algae; *Dunaliella tertiolecta*; *Isochrysis galbana*; *Phaeodactylum tricornutum*; *Tetraselmis suecica*

Introduction

The cultivation of microalgae is part of the biotechnology of growing marine molluscs, crustaceans and fishes. In all types of molluscan aquaculture it is necessary to have a large microalgal biomass available as a source of food for normal development and growth of the cultured species. In fact, production of this biomass is frequently cited as critical to the success of the project and as being a serious impediment to the future development to shellfish aquaculture (De Pauw *et al.*, 1983). The mass culture of certain species of microalgae is considered the major bottleneck in the nursery culturing of molluscs (Persoone & Claus, 1980).

Mass production of microalgae has long been successfully carried out either by using domestic or industrial wastes (De La Noue & De Pauw, 1988; Oswald, 1988) or inorganic compounds, although in rearing of marine molluscs clean nutrient sources are often used (Shelef & Soeder, 1980; Fabregas *et al.*, 1985). These media are generally laboratory preparations, with a relatively high economic cost (Stein, 1973; Soeder, 1980; Fabregas *et al.*, 1984; Fabregas *et al.*, 1985). Different efforts have been carried out for using common fertilizers as microalgal nutrients (Gonzalez-Rodriguez & Maestrini, 1984; Fabregas *et al.*, 1987), but results were not as good as those obtained with the different synthetic algal nutrients (Stein, 1973; Gonzalez-Rodriguez & Maestrini, 1984). Recently, a commercial nutrient for marine microalgae (Algal-1) has been developed by Nutricion Avanzada SA, Santiago de Compostela, Spain.

The use of this commercial nutrient Algal-1 was studied in mass cultures of the marine microalgae *Tetraselmis suecica*, *Dunaliella tertiolecta*, *Isochrysis galbana* and *Phaeodactylum tricornutum*, in comparison with the use of media such as ES-Provasoli, f/2 and Walne medium.

Material and methods

Four different marine microalgae were used: *Tetraselmis suecica*, *Dunaliella tertiolecta*, *Isochrysis galbana* and *Phaeodactylum tricornutum* var. *bicornutum*. They were cultured in seawater filtered through a 0.45 μ m Millipore filter, autoclaved at 120°C for 20 min and enriched with the different culture media: ES-Provasoli, f/2 and Walne (Stein, 1973; Walne, 1974) and Algal-I. This medium was prepared following the manufacturer instructions.

Mass cultures were carried out in a controlled environmental incubator at $18 \pm 1^\circ\text{C}$, 130 $\mu\text{Em/s}$ light from fluorescent lamps and 35‰ salinity. A 12:12 light-dark period was maintained. Cultures had air continuously bubbled through them at a rate of 15 liters/min. The inocula used were: 0.5×10^6 cells/ml for *T. suecica* cultures, 0.75×10^6 cells/ml for *D. tertiolecta* cultures, 2.5×10^6 cells/ml for *I. galbana* cultures and 1.5×10^6 cells/ml for *P. tricornutum* cultures.

Microalgal growth was determined daily by counting culture aliquots in a Coulter Counter model DN.

To obtain growth, velocity doublings/day were calculated:

$$\text{doublings/day} = \frac{\ln N(n) - \ln N(i)}{\ln 2(t_n - t_i)}$$

where t_i and t_n are the initial and final time of the logarithmic phase, both expressed in days, and $N(i)$ and $N(n)$ are the initial and final cellular densities, respectively.

Protein and carbohydrates were measured in the crude extract obtained after collecting the microalgal cells by centrifugation, resuspending them in distilled water and breaking them in an ultrasonic disintegrator. After sonication for 5 min, the extracts were centrifuged again, the pellets were discarded and protein and carbohydrates were measured in the supernatants. Protein was measured by the dye-binding method (Bradford, 1976) and carbohydrates by the phenol-sulfuric acid method as described by Kochert (1978).

Lipids were measured by the charring method of Marsh and Weinstein (1966).

Stationary phases, corresponding to maximum biomass production, were compared by an overall multivariate one-way analysis of variance (ANOVA) ($P \leq 0.05$).

Results and discussion

The marine microalgal species assayed grew in all the culture media used but growth was significantly higher in Algal-I cultures than in the remaining culture media used, for all the microalgal species assayed (Fig. 1). It is well known that the growth of microalgae in batch cultures is often well characterized by the logistic growth function. The growth kinetics model of all the cultures carried out in the present experiments (Fig. 1) fitted a logistic curve (Schanz & Zahler, 1981).

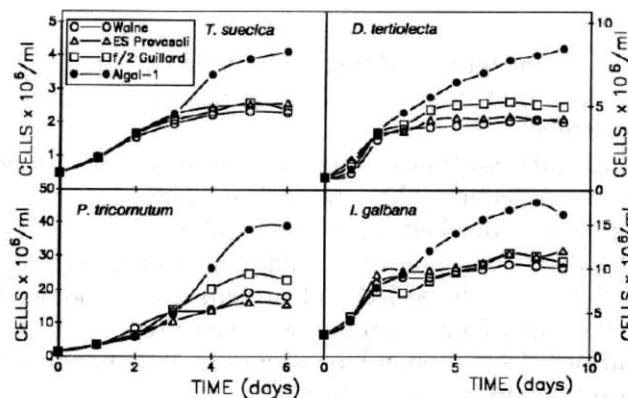


Fig. 1. Growth curve of different marine microalgae grown with different culture media.

The culture medium used affected the velocity of growth of the cultures of the different marine microalgae, but this effect was lower than their effect on biomass production at the end of the stationary phase. Maximum growth rates in the logarithmic phase were between 0.91 and 0.95 doublings/day for *T. suecica*, between 1.04 and 1.58 doublings/day for *D. tertiolecta*, between 0.85 and 1.16 doublings/day for *I. galbana* and between 1.13 and 1.32 doublings/day for *P. tricornutum*. As previously shown, variations in different parameters such as salinity, nutrient concentration or nutrient composition had a greater effect on the final biomass of different microalgal species than on the velocity of growth (Fabregas *et al.*, 1984, 1985).

Maximum cellular densities at the stationary phase were always found in Algal-1 cultures (Fig. 2, Table 1), and these values were always significantly higher than those obtained in the remaining media. These maximum cellular densities were 4.11×10^6 cells/ml for *T. suecica*, 8.45×10^6 cells/ml for *D. tertiolecta*, 16.15×10^6 cells/ml for *I. galbana* and 39.04×10^6 cells/ml for *P. tricornutum*. Cellular densities obtained with the remaining culture media were very similar for *T. suecica*, *D. tertiolecta* and *I. galbana*, with differences ranging between 13% and 23%. However, these variations are higher in *P. tricornutum* cultures, with f/2 cultures showing cellular densities 30% and 52% higher than those obtained in Walne and ES Provasoli media, respectively, although the cellular density reached in f/2 medium only accounted for 58% of that reached in the Algal-1 medium.

Considering the ratio between the maximum cellular density reached in each traditional medium and maximum cellular density reached in Algal-1 for all the species studied (Table 2), ratios near to 0.5 occurred for *Tetraselmis suecica*, *Dunaliella tertiolecta* and *Phaeodactylum tricornutum*, whereas higher ratios appear in *Isochrysis galbana* cultures. Therefore, microalgal yields can be doubled by using algal medium instead of Walne, f/2 or ES-Provasoli media.

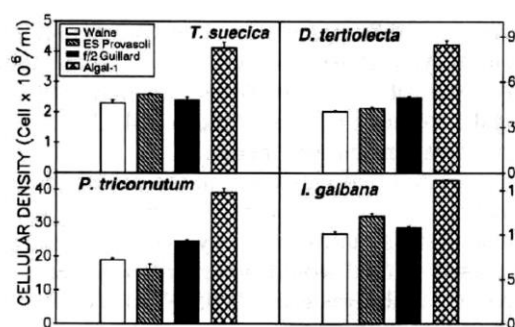


Fig. 2. Maximum cellular density (cells $\times 10^6$ /ml) in the stationary phases of marine microalgae grown with different culture media.

The culture medium used affected the biochemical composition of microalgal cells (Table 1). Protein is the major cellular component for all the species assayed and with all the media proved. Differences in the protein content were higher than those found in the other cellular constituents. Protein/cell ratios obtained in the different traditional culture media were, in all cases, significantly ($P < 0.05$) different to those obtained in the AJgal-1 medium, for all the species assayed (Table 1).

TABLE 1
Cellular Density (Cells $\times 10^6$ /ml) and Chemical Composition (pg/Cell) of Four Marine Microalgae Grown in Different Culture Media

	Cellular density	Protein	Carbohydrates	Lipids
<i>T. suecica</i>				
Walne	2.29	13.31	6.20	7.04
ES	2.58	16.98	6.93	7.22
f/2	2.38	21.75	8.37	7.92
Algal-1	4.11	32.22	8.83	8.65
<i>D. tertiolecta</i>				
Walne	4.04	13.37	13.22 ^a	22.28
ES	4.24	14.88	15.73	23.94
f/2	4.97	13.26	17.91	23.67
Algal-1	8.45	18.82	11.08	18.18
<i>I. galbana</i>				
Walne	10.11	5.17	4.28 ^a	25.95
ES	12.09	7.23	5.21	28.38
f/2	10.81	8.13	5.59	26.82
Algal-1	16.15	9.57	4.28	20.68
<i>P. tricornutum</i>				
Walne	19.01	2.65	6.42 ^a	6.51
ES	16.23	5.21	9.20	6.45
f/2	24.65	3.34	6.90	5.52
Algal-1	39.04	4.20	5.98	5.79

^aNot significantly different of Algal-1 cultures.

Maximum values of protein: cell were obtained in Algal-1 cultures, except for *P. tricornutum* cultures. These maximum values were 32.22 pg/cell for *T. suecica*, 18.82 pg/cell for *D. tertiolecta* and 9.57 pg/cell for *I. galbana*. Contrarily, in *P. tricornutum* cultures, maximum protein/cell concentrations were obtained in Walne medium with

5.21 pg/cell. Therefore, Algal-I cultures produced more cells (Fig. 2) that had more protein than the remaining culture media.

TABLE 2
Ratios Between Values of Cellular Density, Protein/liter Carbohydrates/liter and Lipids/
liter Obtained in Each Medium and Those Obtained in Algal-1 Medium

	Cellular density	Protein	Carbohydrates	Lipids
<i>T. suecica</i>				
Walne	0.55	0.23	0.39	0.45
ES	0.62	0.33	0.49	0.52
f/2	0.57	0.39	0.55	0.53
Algal-1	1.00	1.00	1.00	1.00
<i>D. tertiolecta</i>				
Walne	0.47	0.34	0.56	0.58
ES	0.50	0.40	0.69	0.66
f/2	0.58	0.41	0.93	0.76
Algal-1	1.00	1.00	1.00	1.00
<i>I. galbana</i>				
Walne	0.62	0.34	0.63	0.79
ES	0.74	0.56	0.91	1.03
f/2	0.67	0.57	0.88	0.87
Algal-1	1.00	1.00	1.00	1.00
<i>P. tricornutum</i>				
Walne	0.48	0.31	0.52	0.55
ES	0.41	0.52	0.64	0.46
f/2	0.63	0.50	0.73	0.60
Algal-1	1.00	1.00	1.00	1.00

It has been suggested that variability in the protein content per cell is the most important factor in the use of marine microalgae for animal feeding in aquaculture systems, and any system of mariculture where microalgae are used as feed would be severely affected by variations in their protein content (Fabregas *et al.*, 1985; Wikfors, 1986).

However, maximum values of reserve products (carbohydrates and lipids) never occurred in Algal-I cultures, except for *T. suecica* (Table 1). Maximum carbohydrates/cell values occurred in Algal-1 for *T. suecica* with 8.83 pg/cell; f/2 cultures for *D. tertiolecta* and *I. galbana*, showed 17.91 and 5.59 pg/cell, respectively, and Walne medium for *P. tricornutum* showed 9.20 pg/cell.

Lipids constitute the most constant cellular fraction for all the species in the different culture media (Table 1). Values were between 7.04 and 8.65 pg/cell for *T. suecica*, between 18.18 and 23.94 pg/cell for *D. tertiolecta*, between 20.68 and 28.38 pg/cell for *I. galbana* and between 5.52 and 6.45 for *P. tricornutum*.

Differences in cellular densities and cellular contents provoked higher variations in the values of protein, lipids and carbohydrates obtained per liter of culture.

Maximum values of protein, lipids and carbohydrates per liter of culture always occurred in Algal-I cultures, with significant differences in the values obtained in the remaining cultures (Figs 3-6). Maximum protein concentrations in mg/liter of culture were 132.43 mg/liter for *T. suecica*, 159.04 mg/liter for *D. tertiolecta*, 154.5 for *I. galbana* and 163.88 for *P. tricornutum*, always in Algal-I cultures (Figs 3-6).

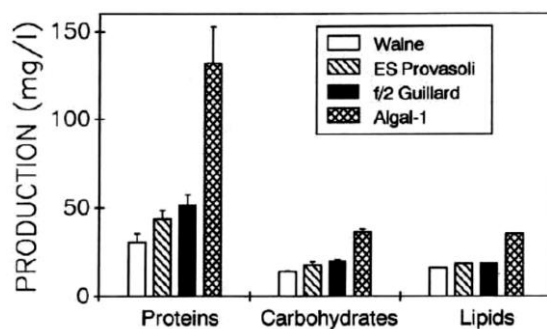


Fig. 3. Maximum protein, lipids and carbohydrates values (mg/liter) obtained in cultures of the marine microalga *Tetraselmis suecica* grown in different culture media.

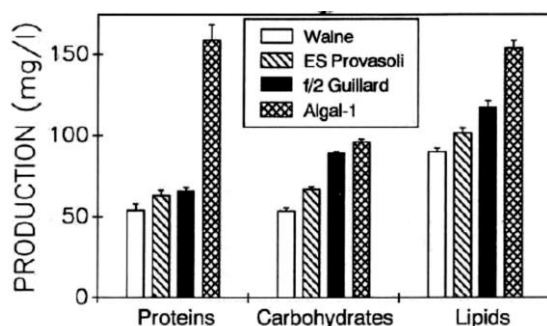


Fig. 4. Maximum protein, lipids and carbohydrates values (mg/liter) obtained in cultures of the marine microalga *Dunaliella tertiolecta* grown with different culture media.

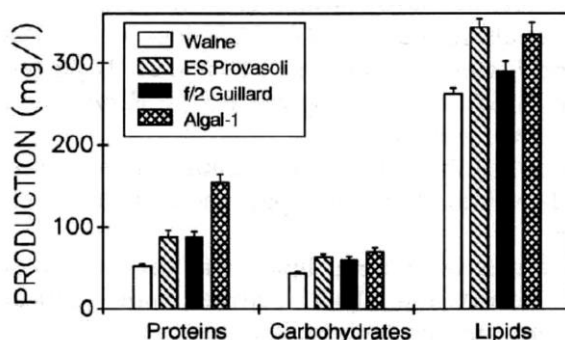


Fig. 5. Maximum protein, lipids and carbohydrates values (mg/liter) obtained in cultures of the marine microalga *Isochrysis galbana* grown with different culture media.

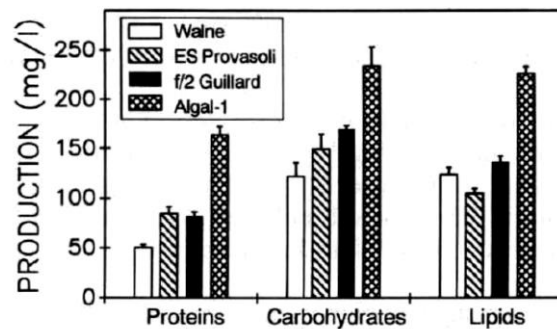


Fig. 6. Maximum protein, lipids and carbohydrates values (mg/liter) obtained in cultures of the marine microalga *Phaedactylum tricornutum* grown with different culture media.

Maximum carbohydrates/liter concentrations also occurred in Algal-1 cultures (Figs 3-6) with values of 36.31 mg/liter for *T. suecica*, 95.75 mg/liter for *D. tertiolecta*, 69.18 mg/liter for *I. galbana* and 233.56 mg/liter for *P. tricornutum*. Maximum lipids concentration per liter of culture occurred in Algal-1 cultures for *T. suecica*, *D. tertiolecta* and *P. tricornutum*, with values of 35.55 mg/liter, 153.66 mg/liter and 225.86 mg/liter, respectively, whereas in *I. galbana* cultures maximum lipid concentration per liter occurred in ES Provasoli (343.12 mg/liter). Variations in carbohydrate and lipid concentrations per liter of culture are lower than those found in protein/liter for all the species assayed (Figs 3-6).

Ratios between protein, carbohydrate and lipid values per liter of culture reached in each traditional medium and those reached in Algal-1 were calculated for all the species studied (Table 2). Ratios between 0.2 and 0.6 were found for protein content; therefore, Algal-1 cultures produced between 2 and 5 times more protein than the remaining cultures. Ratios for carbohydrates and lipids were between 0.4 and 1.0, and differences between traditional culture media and Algal-1 were generally lower than those obtained for proteins.

Biomass yields, as number of cells per ml of culture per day, were always maximum in Algal-1 cultures (Table 1), showing significantly higher values than those obtained in the remaining culture media. These maximum biomass yields were 60×10^4 cells/ml/day for *T. suecica*, 86×10^4 cells/ml/day for *D. tertiolecta*, 152×10^4 cells/ml/day for *I. galbana* and 626×10^4 cells/ml/day for *P. tricornutum*. These values are between 71% and 100% higher than those obtained in the remaining cultures for *T. suecica*, between 83% and 132% for *D. tertiolecta*, between 42% and 79% for *I. galbana* and between 62% and 154% for *P. tricornutum*.

Maximum yields in protein, carbohydrate and lipid per liter of culture per day were obtained in Algal-1 cultures for all the species assayed, excepting the yield in lipids for

I. galbana cultures, which reached the maximum value in ES-Provasoli cultures (31-33 mg/liter/day) without significant differences from Algal-1 cultures (30-30 mg/liter/day) (Table 3)_

Yields in protein showed higher differences than those found both in carbohydrates and lipids (Table 2)_ Daily protein yields in Algal-1 medium were 20-10 mg/liter/day for *T. suecica* cultures, 15-66 mg/liter/ day for *D. tertiolecta*, 14-90 mg/liter/day for *I. galbana* and 26-06 mg/ liter/day for *P. tricornutum*, whereas daily protein yields with the traditional culture media used were between 3-10 and 6-65 mg/liter/day for *T. suecica* cultures, between 3-99 and 5-32 mg/liter/day for *D. tertiolecta* cultures, between 3-54 and 7-49 mg/liter/day for *I. galbana* and between 7-42 and 12-73 mg/liter/day for *P. tricornutum* cultures.

TABLE 3
Yields in Cells (Cells $\times 10^6$ /per ml/day), Protein, Carbohydrates and Lipids (mg/liter/day) of Four Marine Microalgae Grown in Different Culture Media

	Cellular density	Protein	Carbohydrates	Lipids
<i>T. suecica</i>				
Walne	0.30	3.10	1.58	1.30
ES	0.35	5.33	2.19	1.72
f/2	0.31	6.65	2.53	1.75
Algal-1	0.60	20.10	5.26	4.54
<i>D. tertiolecta</i>				
Walne	0.37	3.99	5.01	8.51
ES	0.39	5.00	6.49	9.79
f/2	0.47	5.32	8.97	11.58
Algal-1	0.86	15.66	9.72	15.58
<i>I. galbana</i>				
Walne	0.85	3.54	2.68	22.35
ES	1.07	7.44	4.88	31.33
f/2	0.92	7.49	4.59	25.42
Algal-1	1.52	14.90	5.56	30.30
<i>P. tricornutum</i>				
Walne	2.92	7.42	18.30	19.37
ES	2.46	13.13	22.86	16.19
f/2	3.86	12.73	26.32	21.43
Algal-1	6.26	26.06	36.89	36.39

Yields in carbohydrates varied between 1.58 and 5.26 mg/liter/day in *T. suecica* cultures, between 5.01 and 9.72 mg/liter/day for *D. tertiolecta*, between 2.68 and 5.56 mg/liter/day for *I. galbana*, and between 18.30 and 36.89 mg/liter/day for *P. tricornutum*. Minimum yields in carbohydrates were obtained, for all the species assayed, in Walne cultures, which were about 50% lower than those obtained in Algal-1 cultures (Table 3).

With regards to lipids, the yields varied between 1.30 and 4.54 mg/liter/day in *T. suecica* cultures, between 8.51 and 15.58 mg/liter/day for *D. tertiolecta*, between 22.35 and 31.33 mg/liter/day for *I. galbana*, and between 16.19 and 36.39 mg/liter/day for *P. tricornutum* (Table 2).

In mariculture it has already been proved that differences in growth media affect the growth and gross chemical composition of algal food sources (Myklestad & Haug, 1972; Shifrin & Chisholm, 1980; Fabregas *et al.*, 1985, 1986a, b), which alone can account for differences in algal nutritional value to commercially important marine herbivore species (Wikfors, 1986).

ES-Provasoli, f/2 and Walne media are widely used in marine microalgae mass culture. Results showed better yields in Algal-1 cultures than yields obtained with these traditional culture media. Algal-1 was found to be a cheap nutrient supply and shows great feasibility for the preparation of microalgal culture media.

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